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Simple and Sensitive Procedure for Screening Yeast Mutants That Lyse at Nonpermissive Temperatures

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After mutagenesis, surviving yeast cells are grown on plates at 25 C and later exposed to 37 C. The plates are then overlaid with a soft agar containing *p*-nitrophenylphosphate at pH 9.7. Lysed cells liberate alkaline phosphatase which gives rise to a yellow color on and around colonies.

In the course of a study on septum formation in yeast, it became necessary to obtain temperature-sensitive mutants that would lyse at nonpermissive temperatures. Methods for the visualization of phosphatase activity have been reported for bacteria (3, 5) and fungi (1) in which the enzyme can be detected in intact cells. The procedure presented here is based on the fact that alkaline phosphatase is an intracellular enzyme in yeast (6, 7). When yeast colonies are overlaid with a soft agar containing a chromogenic phosphatase substrate and an alkaline buffer, only those colonies containing lysed cells give rise to a colored halo.

In a typical experiment, 3.5×10^8 cells of *Saccharomyces cerevisiae* S288C (ATCC 26108) in 1 ml were treated for 50 min at 30 C with 30 μl of ethyl methane sulfonate, an amount sufficient to kill about 80% of the cells. After centrifuging, the cells were washed several times with water and suspended in water. Suitable dilutions were immediately spread on 100 YEPD (2) plates, each plate receiving 50 to 80 survivors. After 3 days at room temperature, the colonies had attained a diameter of 1 to 3 mm, and the plates were transferred to 37 C overnight. A solution containing 0.5% Noble agar (Difco), 0.05 M glycine hydrochloride at pH 9.7, and 10 mM sodium *p*-nitrophenylphosphate was prepared as follows: agar and the glycine buffer were autoclaved separately and then mixed. A 0.1 M solution of *p*-nitrophenylphosphate was filter-sterilized and stored at −20 C. Before use, the agar was melted by heating briefly in boiling water and then cooled and maintained at 40 C. *p*-Nitrophenylphosphate was added, and 3.3 ml of the liquid mixture were pipetted onto each plate. Between 15 and 60 min after the overlay, colonies containing lysed cells acquire a yellow color and a yellow halo gradually spreads out from them. Samples were picked up with a loop and observed under the microscope. The colonies with the desired morphology (in our case, lysed pairs of mother cell and bud in which both cells were of similar size) were transferred with the loop to another plate for isolation and further study. In experiments resulting in the appearance of 6,000 to 8,000 colonies, about 150 yellow colonies were observed, of which 5 or 6 had the required morphology.

The appearance of lysing colonies is shown in Fig. 1A. As seen in Fig. 1B, wild-type colonies do not give rise to any halo under the same conditions, even after prolonged incubation. Thus, the acidic phosphatase contained in the periplasmic space and readily detected in intact cells (4, 6, 7) does not interfere in this case because of the high pH of the buffer in the overlay.

The method is very rapid because replica-plating is not required. Even in a mutant yielding a strong positive reaction, enough cells remain intact to permit recovery of the strain (Fig. 2), probably because many cells in the colony are not growing and dividing at any specific time. However, replica-plating can be used if so desired and is recommended to obtain mutants which are osmotically sensitive at the nonpermissive temperature. In this case, colonies grown at room temperature should be replica-plated onto media with or without an osmotic stabilizer, and after further growth at 25 C, the plates should be transferred to 37 C and later submitted to the enzymatic test.

The method is exceedingly sensitive because, given sufficient time, even very small amounts of the phosphatase will produce enough *p*-nitrophenol to yield a detectable color. In such cases several fields might be scanned in the micro-

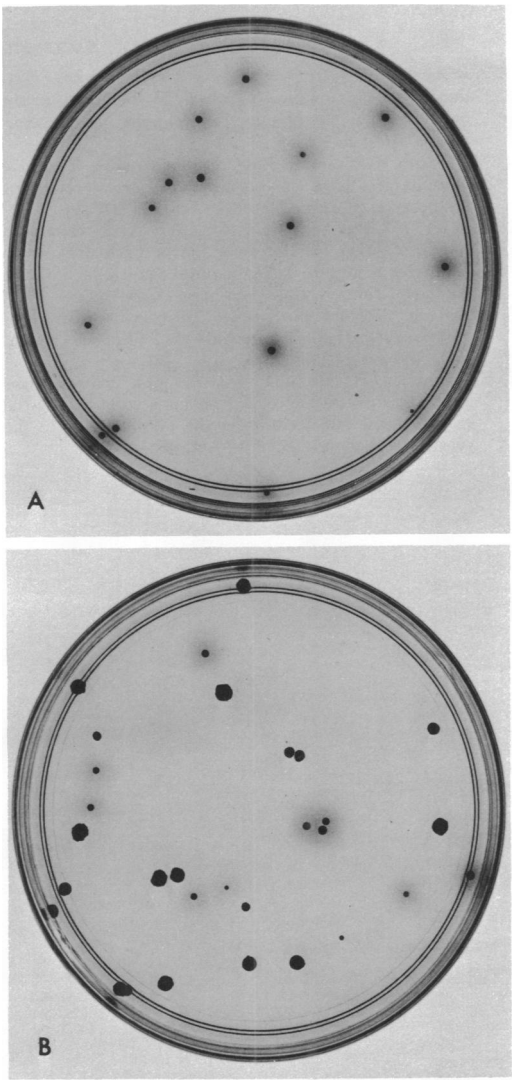

FIG. 1. *Appearance of plates after incubation with p-nitrophenylphosphate under the conditions described in the text. (A) Colonies of lysis mutant L-2-18. (B) Mixture of L-2-18 and wild type. The wild-type colonies, devoid of colored halo, are larger than those of the mutant, because the generation time of the latter is about 60% greater.*

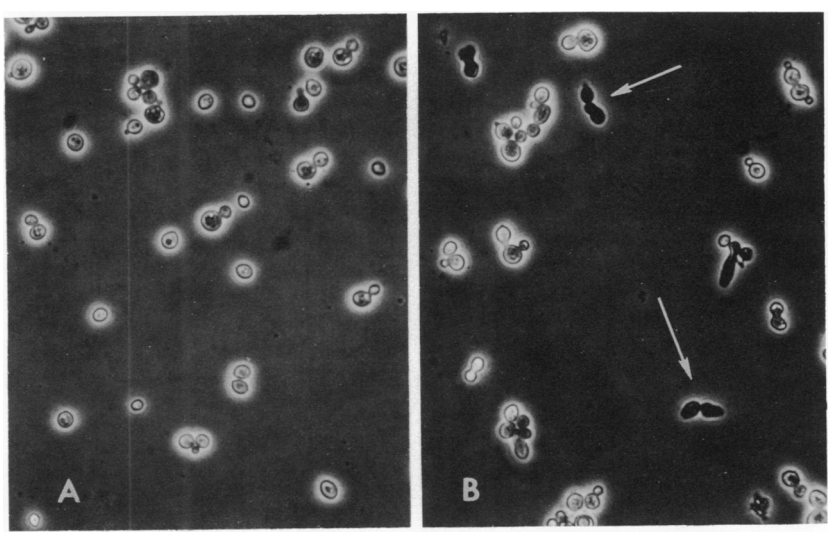

FIG. 2. *Phase-contrast photographs of cells picked from colonies of mutant L-2-18 exposed to 25 C (A) or to 25 C followed by incubation at 37 C (B). In the latter case lysed cells are visible (arrows).* ×430.

scope before finding a lysed cell. Because of this high sensitivity, mutants which are slightly leaky at 25 C will give a marginally positive reaction after prolonged contact with the substrate, even without previous incubation at 37 C.

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